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PATENT TRADEMARK OFFICE

Docket No: 4058/1G811US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Arnold, **FRANCIS**; Petrounia, **IONNA**; Sun, **LIANHONG**

Serial No.: 09/722,602

Art Unit: 1652

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Filed: 11/27/00

Examiner: Y. Pak

For: **DIRECTED EVOLUTION OF OXIDASE ENZYMES**

PENDING CLAIMS

63. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and a mutation in at least one amino acid aligned with an amino acid selected from the group consisting of A3, S10, M70, P136, G195, T218, L312, V494, C515, N535, N537, S610, N413 and S550 of the wild-type galactose oxidase.

64. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and at least one of the amino acid mutations corresponding to S10P, M70V,

G195E, V494A, C515S, N535D, N537D and N413D of the wild-type galactose oxidase.

65. (Twice amended) The isolated variant of claim 64, which has the amino acid mutation corresponding to N537D of the wild-type galactose oxidase.

66. (Twice amended) The isolated variant of claim 64, which has the amino acid mutation corresponding to V494A of the wild-type galactose oxidase.

67. (Twice amended) The isolated variant of claim 66, further comprising the amino acid mutation corresponding to C515S of the wild-type galactose oxidase.

68. (Twice amended) The isolated variant of claim 66, further comprising the amino acid mutation corresponding to S10P of the wild-type galactose oxidase.

69. (Twice amended) The isolated variant of claim 66, further comprising a silent mutation at a position corresponding to P136 of the wild-type galactose oxidase.

70. (Twice amended) The isolated variant of claim 68, further comprising a silent mutation at a position corresponding to P136 of the wild-type galactose oxidase.

71. (Twice amended) The isolated variant of claim 66, further comprising the amino acid mutation corresponding to G195E of the wild-type galactose oxidase.

72. (Twice amended) The isolated variant of claim 71, further comprising a silent mutation in at least one of positions corresponding to A3 and P136 of the wild-type galactose oxidase.

73. (Twice amended) The isolated variant of claim 66, further comprising the amino acid mutation corresponding to N535D of the wild-type galactose oxidase.

74. (Twice amended) The isolated variant of claim 73, further comprising a silent mutation in at least one of positions corresponding to P136, L312, and T218 of the wild-type galactose oxidase.

75. (Twice amended) The isolated variant of claim 66, further comprising the amino acid mutation corresponding to M70V of the wild-type galactose oxidase.

76. (Twice amended) The isolated variant of claim 75, further comprising a silent mutation at a position corresponding to P136 of the wild-type galactose oxidase.

77. (Twice amended) The isolated variant of claim 64, which has the amino acid mutations corresponding to S10P, M70V, G195E, V494A and N535D of the wild-type galactose oxidase.

78. (Twice amended) The isolated variant of claim 77, further comprising a silent mutation at a position corresponding to P136 of the wild-type galactose oxidase.

79. (Twice amended) The isolated variant of claim 64, which has the amino acid mutation corresponding to N413D of the wild-type galactose oxidase.

80. (Three times amended) The isolated variant of claim 79, further comprising a silent mutation at a position corresponding to S550 of the wild-type galactose oxidase.

81. (Twice amended) The isolated variant of claim 66, further comprising the amino acid mutation corresponding to N413D of the wild-type galactose oxidase.

82. (Twice amended) The isolated variant of claim 81, further comprising a silent mutation in at least one of a position corresponding to S550 and S610 of the wild-type galactose oxidase.

83. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and a mutation in at least one amino acid aligned with an amino acid selected from the group consisting of A3, S10, M70, P136, T218, L312, C515, N535, N537, S550, S610, and N413 of the wild-type galactose oxidase.

84. (Twice amended) The isolated variant of claim 83, further comprising at least one amino acid mutation corresponding to a mutation selected from the group consisting of G195 and V494 of the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

85. (Twice amended) The isolated variant of claim 83, wherein the mutation is selected from a mutation corresponding to at least one of the group consisting of S10P, M70V, N413D C515S, N535D, and N537D of wild-type galactose oxidase.

86. (Twice amended) The isolated variant of claim 85, further comprising at least one amino acid mutation corresponding to a mutation selected from the group consisting of G195E and V494A of wild-type galactose oxidase.

87. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and a mutation in an amino acid corresponding to N537 of the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

88. (Twice amended) The isolated variant of claim 87, wherein the mutation is N537D.

89. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and mutations in amino acids corresponding to V494 and C515 of the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

90. (Twice amended) The isolated variant of claim 89, wherein the mutations are V494A and C515S.

91. (Amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and mutations in amino acids corresponding to V494 and P136 of the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

92. (Twice amended) The isolated variant of claim 91, wherein the V494 mutation is V494A.

93. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and mutations in amino acids corresponding to V494, P136, and S10 of the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

94. (Twice amended) The isolated variant of claim 93, wherein the V494 mutation is V494A, and the S10 mutation is S10P.

95. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and mutations in amino acids corresponding to V494, P136, G195, and A3 of the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

96. (Twice amended) The isolated variant of claim 95, wherein the V494 mutation is V494A, and the G195 mutation is G195E.

97. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and mutations in amino acids corresponding to V494, P136, L312, N535, and T218 of the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

98. (Twice amended) The isolated variant of claim 97, wherein the V494 mutation is V494A, and the N535 mutation is N535D.

99. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type galactose oxidase from *D. dendroides* and mutations in amino acids corresponding to V494, P136, and M70 of the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

100. (Twice amended) The isolated variant of claim 99, wherein the V494 mutation is V494A, and the M70 mutation is M70V.

101. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and mutations in amino acids corresponding to V494, S10, P136, M70, G195, and N535 of the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

102. (Twice amended) The isolated variant of claim 101, wherein the V494

mutation is V494A, the S10 mutation is S10P, the M70 mutation is M70V, the G195 mutation is G195E, and the N535 mutation is N535D.

103. (Amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and a mutation in an amino acid corresponding to N413 of the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

104. (Twice amended) The isolated variant of claim 103, wherein the mutation is N413D.

105. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and a mutation in amino acids corresponding to N413 and S550 of the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

106. (Twice amended) The isolated variant of claim 105, wherein the N413 mutation is N413D.

107. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type galactose oxidase *D. dendroides* and a mutation in amino acids corresponding to N413, S550 and V494 of the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.



108. (Twice amended) The isolated variant of claim 107, wherein the N413 mutation is N413D, and the V494 mutation is V494A.

109. (Twice amended) An isolated galactose oxidase variant which has at least 60% amino acid sequence identity to a wild-type *D. dendroides* galactose oxidase and mutations in amino acids corresponding to N413, S550, V494, and S610 of the wild-type galactose oxidase, and wherein the variant has improved D-galactose oxidation activity as compared to the wild-type galactose oxidase.

110. (Amended) The isolated variant of claim 109, wherein the N413 mutation is N413D, and the V494 mutation is V494A.

111. (Allowed) An isolated galactose oxidase having an amino acid sequence selected from the group consisting of SEQ ID NOS: 10-21.

112. (New) The isolated variant of any one of claims 63, 64, 83, 87, 91, 93, 95, 97, 99, 101, 103, 105, 107, and 109, having at least 60% amino acid sequence identity to the wild-type galactose oxidase, wherein the wild-type galactose oxidase has the sequence of SEQ ID NO:10 without the N537D mutation.